IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2663
Steven M. RUBEN	Art Unit: 1644
Appl. No.: 10/662,429	Examiner: HUYNH, PHUONG N.
Filed: September 16, 2003	Atty. Docket: 1488.1890003/EJH/SAC
For: **Apoptosis Inducing Molecule I**	

Declaration of Timothy Coleman
Ruben Exhibit #64

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Paper No. _____

Filed on Behalf of Party Ruben

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Sally Gardner Lane)

STEVEN M. RUBEN

Junior Party,
(Application 08/816,981),

v.

STEVEN R. WILEY and RAYMOND G. GOODWIN

Senior Party,
(Patent No. 5,763,223).

Patent Interference No. 105,077

DECLARATION OF TIMOTHY A. COLEMAN

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I, Timothy A. Coleman, hereby declare and state as follows:

1. From March 1993 to November 2001, I was employed by Human Genome Sciences, Inc. (HGS). During the time period discussed in paragraphs 2 to 8 below, I held the position of Scientist in the HGS Protein Expression and Purification Department, working under the supervision of Dr. Reiner Gentz.

2. While employed by HGS, I was the direct supervisor of Solange Gentz, who was known as Solange Lima at that time. In this role, I reviewed, signed, and dated Solange Gentz's laboratory notebook number 279 (RE69) according to HGS practice at that time, as follows:

Page	Date
75	October 13, 1995
93	November 28, 1995
99	December 19, 1995
105	February 1, 1996
115	February 28, 1996

3. Exhibit RE65 contains certain pages of my laboratory notebook #258 in which I recorded experiments that I performed. These pages outline a pulse-labeling experiment in insect Sf9 cells that I started on June 12th, 1995 to assess the recombinant expression of AIM-1 protein in these cells. The pulse-labeling experiment included infection of Sf9 cells with viruses carrying one of two AIM-I expression constructs derived from clone HTPAN08, to which I refer in my laboratory notebook as FAS-51 and FAS-185, that I had obtained from Solange Gentz (then known as Solange Lima). Details of these expression constructs are described in Solange Gentz's notebook number 279 (RE69), which I reviewed, signed, and dated according to HGS practice at that time.

4. It is my belief that the protocol I used in the pulse-labeling experiment started on June 12, 1995 was substantially as follows:

- Seed approximately 80 to 85% confluent Sf9 cells into a 12 well plate at a density of approximately 8 x 10^5 cells/well in Grace's Insect Medium (Gibco Cat. 11595-022) + 10% HIFBS (heat-inactivated fetal bovine serum) + 1% Penicillin-Streptomycin. Incubate at 27° C with 90% humidity overnight.
- The next day, infect each well with 7-10 µl recombinant baculoviruses. The other three wells are used for controls as follows:
    a. Uninfected well
    b. Negative control (recombinant virus without cDNA insert)
    c. Positive control (Known expressed virus)
- Return the 12 well plate to the incubator.
- After 5 hours inoculation, the medium is replaced with 1.0 ml of Sf900II serum-free medium depleted for methionine & cysteine (Special formulation order from Gibco) in order to starve the cells.
- At day 3 post infection, the culture medium from each well is replaced with 0.5 ml of Sf900 II SFM (-Met -Cys) containing 40 µCi each ^{35}S-methionine (Amersham Life Science, Cat. SJ 1515) & ^{35}S-cysteine (Amersham Life Science, Cat. SJ 232).
- Return the 12 well plate to the incubator.
- Infected cells are labeled for 20-24 hours after which the culture medium is removed and clarified by centrifugation @ 14,000 rpm for 5 minutes

(Supernatant). The cells are lysed in the 12 well plate by addition of 200 µl lysis buffer (20 mM Hepes, pH 7.9; 130 mM NaCl; 0.2 mM EDTA; 0.5 mM DTT; 0.5% (v/v) NP-40) and then diluted up to 1.0 ml with dH_20 (Cell extract).

- 30 µl of each supernatant and cell extract combined with 15 µl 3X SDS sample buffer (loaded into a 14 well comb) are resolved by 0.1% SDS-15% PAGE, running at 14 Watts/2 hours 30 minutes
- Protein gels are done as follows:
  - Stained approximately 20 minutes
  - Destained approximately 1 hour
  - Amplified approximately 30 minutes (Amplify, Amersham, NAMP 100)
  - Dried approximately 1 hour 40 minutes/80° C
  - Autoradiographed (24 hours-96 hours exposure)

5. Briefly, this protocol involved infecting Sf9 cells with recombinant secondary virus (*i.e.*, plaque-purified and amplified in small volumes) for 2-3 days. For each of FAS-51 and FAS-185, I used two viral isolates (referred to in my notebook as -1 and -2) in the pulse labeling experiment I initiated on June 12, 1995. The lane labeled with a circled "2" in my notebook appears to show labeling of uninfected Sf9 cells (Exhibit RE65, pages 110-111).

6. The viral infection of Sf9 cells is followed by removing the growth media and adding a serum-free medium containing ^{35}S-methionine and ^{35}S-cysteine. The label was allowed to incorporate overnight, and then supernatant and cell extracts were

prepared for SDS-PAGE analysis. Thus, in the experiments I initiated on June 12, 1995, the infections and labeling would represented continuous work through at least June 16, 1995.

7. SDS-PAGE gels of extracts of the pulse-labeled Sf9 cells (including cells infected with the viruses harboring the AIM-I constructs and the wild type virus control) were run on June 16, 1995 and allowed to dry overnight, *i.e.*, until June 17, 1995.

8. The dried gel was subject to autoradiography for different periods of time to visualize the labeled protein bands. RE66 shows two different exposures of the gel, one of 42 hours and one of 100 hours. The 42-hour exposure was developed on June 19, 1995, and thus the dried gel must have been placed on film June 17, 1995 and exposed to the film through June 19, 1995, when the film was developed. Although the 100-hour exposure was undated, it was my customary practice at the time to immediately re-expose the dried gel to film if an initial shorter exposure did not produce a sufficient film image. Thus, it is my belief that the 100-hour exposure was initiated on June 19, 1995 and continued through June 23, 1995, at which time I would have developed the film for the 100-hour exposure.

9. The autoradiogram of the 42-hour exposure is labeled to indicate the source of the proteins in each lane. The five lanes entitled HTPAN08 Fas Ligand represent cell extracts from Sf9 cells infected with the two virus preparations containing the FAS-51 construct, apparently uninfected Sf9 cells, and Sf9 cells infected with the two virus preparations containing the FAS-185 construct, respectively. The autoradiogram of the 100-hour exposure indicates an approximately 31 kilodalton band present in the lane corresponding to the FAS-185-expressing cells (RE66), which is

approximately the predicted molecular weight of the protein encoded by this construct. This band is absent from the apparently-uninfected Sf9 cells. The 42 hour and 100 hour film exposures (RE66) were stored in hand-made sleeves on pages 112-113 of my notebook (RE65).

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

Date: 6/23/04

Timothy A. Coleman